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Diversity of cleavage patterns of *Salmonella* 23S rRNA

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The recent discovery of the phenomenon that some prokaryotes fragment their 23S rRNA during post-transcriptional processing of precursor rRNA has been shown to be particularly prevalent among strains and species of *Salmonella*. Some strains of *Salmonella* cleaved 23S rRNA at multiple sites producing several fragments. The cleavage patterns of 23S rRNA differed among *Salmonella* strains and sometimes among the rRNA operons in the same strain. Fragmentation of 23S rRNA was not observed in strains of the closely related species *Escherichia coli*. Fragmentation of 23S rRNA occurred in *Brucella* and *Agrobacterium* but the cleavage pattern was not as diverse as that demonstrated in *Salmonella*. Introduction of cleavage sites into precusor 23S rRNA of *Salmonella* is probably a recent evolutionary event.

Introduction

Ribosomes from *Escherichia coli* are composed of three species of rRNA and 52 different ribosomal proteins (Wittmann, 1982). The ribosome plays multiple roles. Among others, it is the site of peptidyl transferase activity; it keeps mRNAs in proper register to recognize charged tRNAs; it participates in proper translational initiation and termination, and it interacts with at least seven accessory proteins (Shine & Dalgarno, 1974; Weissbach, 1980; Gold *et al.*, 1981). Ribosomes are also transducers of metabolic signals in response to shortage of amino acids, and probably growth rate as well as exposure to temperature extremes. rRNA plays an active functional role as well as a structural role (Woese *et al.*, 1980; Noller, 1984). For example, resistance to the antibiotics chloramphenicol, erythromycin and paromomycin can result from point mutations in rRNA genes (Noller, 1984). Owing to its multiple critical roles, rRNA has been highly conserved during evolution making it an excellent index of phylogenetic relationships among diverse organisms (Pace *et al.*, 1986). A large number of the small subunit rRNAs from different organisms have been sequenced and this information has been used to construct a comprehensive phylogenetic tree showing the relationship of all living things.

The rRNAs in most prokaryotic cells are transcribed from redundant operons as single precursor molecules, which are then processed by several RNAases into mature 16S, 23S and 5S rRNA components (Noller, 1984). The rRNAs of typical eukaryotic cells are also processed during maturation, but with one extra cleavage site four fragments, 26–28S, 18S, 5·8S and 5S, are produced. The extra cleavage site lies in the eukaryotic equivalent of the prokaryotic 23S rRNA (Doolittle & Pace, 1971; Jacq, 1981).

Despite the highly conserved nature of rRNAs they do vary with respect to size and number of cleavage sites. Besides the well known variation in size of mitochondrial rRNAs (Noller, 1984), some protozoa contain in their cytoplasmic ribosomes smaller rRNAs (Gundersom & Sogin, 1986; Edlind & Chakraborty, 1987; Vossbrinck *et al.*, 1987) and certain eukaryotes, including protostomes, protozoa and some coelenterates (Ishikawa, 1977; Clark & Gerbi, 1982; Ware *et al.*, 1985), contain one or more short stretches of spacer sequence in their 28S or 5·8S rRNA molecules which are removed during maturation, resulting in fragmented rRNA molecules (Pavlakis *et al.*, 1979; Jordan *et al.*, 1980; Ware *et al.*, 1985; Campbell *et al.*, 1987; Spencer *et al.*, 1987; Hsu *et al.*, 1990). At least one extra cleavage site has been reported in the large subunit of certain prokaryotic rRNAs: in *Leptospira*, *Anacystis*, *Micrococcus*, *Haemophilus*, *Synechococcus*, *Agrobacterium*, *Rhodobacter* and in two species of *Salmonella* (*S. typhimurium* and *S. arizonae*) (Marrs & Kaplan, 1970; Grienenberger *et al.*, 1972; Siebens & Trench, 1978; Smith *et al.*, 1988; Boom *et al.*, 1990;

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Burgin *et al.*, 1990; Hsu *et al.*, 1990). Although the origin of these extra cleavage sites is still unclear, they are apparently not within the spacer sequences that are responsible for the fragmentation of the eukaryotic rRNAs.

In this study we report the high frequency and diversity of cleavage patterns among and within strains of *Salmonella*. The nature of these cleavage sites is clearly different from those in other prokaryotes.

Methods

Bacterial strains. rRNA of various *Salmonella* strains and isolates (*S. typhimurium*, *S. dublin*, *S. montevideo*, *S. typhisuis*, *S. reading*, *S. infantis*, *S. enteritidis*, *S. choleraesuis*, *S. hadar*, *S. albany*, *S. kentucky*, *S. heidelberg*, *S. newport* and *S. ohio*), *Escherichia coli* strains (JM83, DH5α and 30 clinical isolates), *Brucella* strains (*B. suis*, *B. ovis*, *B. canis* and field strains and strain 19 of *B. abortus*) and *Agrobacterium* strains (*A. viscosum*, *A. tumefaciens* and *A. radiobacter*) were examined in this study. All strains were grown on sheep-blood agar plates at 37 °C, except *Agrobacterium* strains which were grown on LB agar plate at 30 °C.

rRNA extraction and gel electrophoresis. rRNA was extracted by lysing cells with SDS solution and fractionating the lysate by electrophoresis on agarose gels as previously described (Hsu *et al.*, 1990).

Northern blot hybridization. Standard procedures for Northern blot hybridization were followed (Maniatis *et al.*, 1982), except that non-

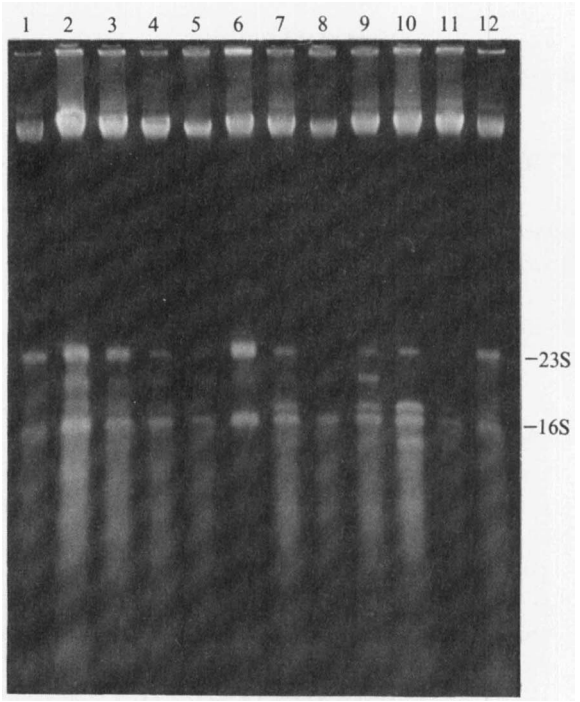

Fig. 1. Electrophoresis patterns of rRNA molecules of different *Salmonella* strains and *E. coli* on a non-denaturing agarose gel. Lanes: 1, *E. coli* JM83; 2, *S. choleraesuis*; 3, *S. hadar*; 4, *S. albany*; 5, *S. kentucky*; 6, *S. typhisuis*; 7, *S. heidelberg*; 8, *S. newport*; 9, *S. infantis*; 10, *S. typhimurium* (field isolate D9001362); 11, *S. typhimurium* (field isolate D8902865); 12, *E. coli* JM83.

Table 1. *Cleavage patterns of Salmonella 23S rRNA*

	Bacterial strain	Hetero-zygous*	Number of cleavage sites†	Fragments generated‡						
				23S (2·9 kb)	21S (2·4 kb)	19S (1·8 kb)	18S (1·7 kb)	17S (1·6 kb)	14S (1·2 kb)	13S (1·1 kb)
A	*S. typhimurium* (isolates K871208, D8902865)	No	1	−	−	−	+++	−	+++	−
B	*S. typhimurium* (isolate D8805467)	Yes	2	−	+	−	+++	−	+++	−
C	*S. typhimurium* (isolate D9001362)	Yes	1	+	−	−	+++	−	+++	−
D	*S. dublin*	Yes	2	+++	−	−	+	+	−	−
E	*S. montevideo*	Yes	3	+++	+	−	+	+	−	−
F	*S. typhisuis*	Yes	1	+++	+	−	−	−	−	−
	S. reading	Yes	1	+++	+	−	−	−	−	−
	S. enteritidis	Yes	1	+++	+	−	−	−	−	−
G	*S. choleraesuis*	Yes	3	+++	++	−	+	−	+	+
	S. kentucky	Yes	3	+++	++	−	+	−	+	+
	S. heidelberg	Yes	3	+++	++	−	+	−	+	+
	S. newport	Yes	3	+++	++	−	+	−	+	+
	S. ohio	Yes	3	+++	++	−	+	−	+	+
H	*S. hadar*	Yes		+++	++	+	+	−	−	+
	S. albany	Yes	3	+++	++	+	+	−	−	+
I	*S. infantis*	Yes	3	++	++	−	++	−	++	++
	E. coli	No	0	+++	−	−	−	−	−	−

* Differences among rRNA genes with respect to cleavage site(s).
† Minimum number.
‡ Number of '+' symbols indicates relative abundance of fragment.

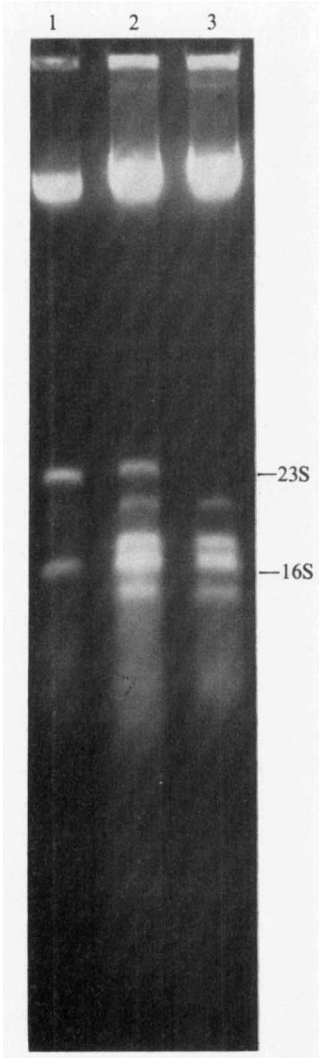

Fig. 2. Electrophoresis of the mixed culture of *E. coli* and *S. typhimurium*. Lanes: 1, *E. coli* JM83 lysed alone; 2, *E. coli* JM83 mixed with S. *typhimurium* at a 1:1 ratio before lysis; 3, *S. typhimurium* lysed alone.

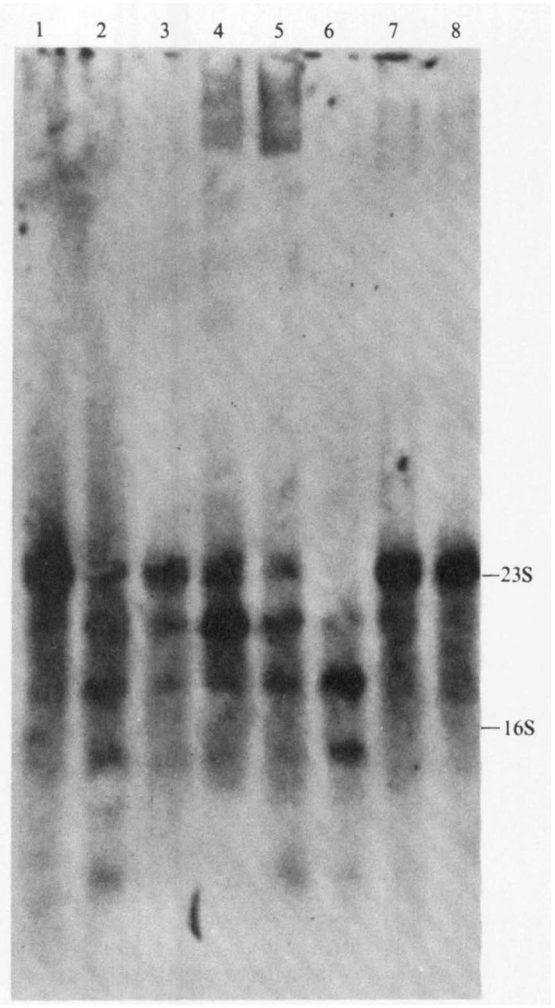

Fig. 3. Northern blot hybridization of a ^{32}P-labelled 23S rRNA-specific probe to rRNA fragments of different *Salmonella* and *E. coli* strains. Lanes: 1, *E. coli* JM83; 2, *S. infantis*; 3, *S. albany*; 4, *S. newport*; 5, *S. ohio*; 6, *S. typhimurium* (field isolate 08902865); 7, *S. enteritidis*; 8, *E. coli* DH5α.

denaturing agarose gels were used. The rRNAs fractionated on agarose gels were transferred to a nitrocellulose membrane using 20 × SSC (1 × SSC is 0·15 M-NaCl, 0·015 M-sodium citrate. The membrane was subsequently hybridized with a 23S rRNA-specific and a 16S rRNA-specific radioactively labelled probe, respectively. The probes were subcloned from plasmid pC6 containing *E. coli* 23S and 16S rRNA sequences.

Results

Cleavage patterns of Salmonella 23S rRNA

Most of the *Salmonella* strains we examined contained fragmented 23S rRNAs (Fig. 1), the patterns of which were highly variable among strains suggesting that cleavage sites have been inserted in rRNA genes at several locations (Table 1). The size of these subfragments ranged from 13S to 21S. In some *S. typhimurium* isolates, no 23S rRNA was observed, indicating all seven copies of rRNA genes contained cleavage sites. In others the copies varied; both intact and fragmented 23S rRNA were present. To eliminate the possibility that the fragmentation was an artifact of our experimental procedures, 30 independent *E. coli* clinical isolates were examined with the same procedures. None of these *E. coli* isolates contained fragmented rRNA (data not shown). Furthermore, when mixed culture of *E. coli* and *S. typhimurium* was examined, the 23S rRNA of *E. coli* was found intact in the presence of fragmented 23S rRNA from *S. typhimurium* (Fig. 2).

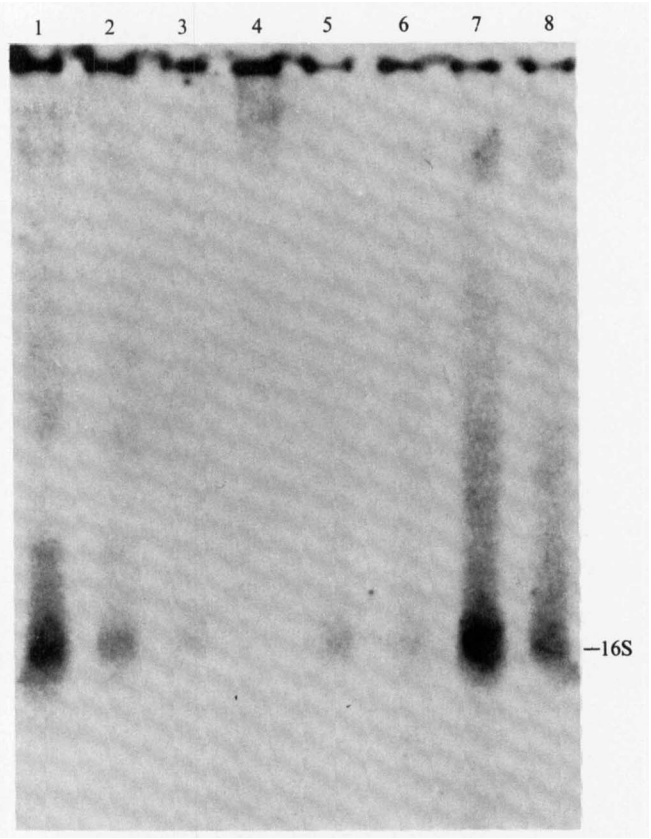

Fig. 4. Northern blot hybridization of a ^{32}P-labelled 16S rRNA-specific probe to rRNA fragments of different *Salmonella* and *E. coli* strains. Lanes: 1, *E. coli* JM83; 2, *S. infantis*; 3, *S. albany*; 4, *S. newport*; 5, *S. ohio*; 6, *S. typhimurium* (field isolate 08902865); 7, *S. enteritidis*; 8, *E. coli* DH5α.

Northern blot hybridization

In order to verify that the RNA fragments we observed were derived from 23S rRNAs, Northern blot hybridization was employed. The result showed that 23S, 21S, 19S, 18S, 17S, 14S and 13S fragments hybridized to the 23S-specific probe (Fig. 3); only the 16S fragment hybridized to the 16S-specific probe (Fig. 4). Thus, only 23S rRNAs were fragmented; the 16S rRNAs remained intact.

Discussion

Extra cleavage sites in rRNAs appear to occur almost randomly among *Salmonella* strains, sometimes even among different rRNA genes in the same strain. No extra cleavage sites were detected in the 30 clinical isolates of *E. coli* that we examined. It has been proposed by others that fragmentation of prokaryotic rRNA is caused by a random event: insertion into rRNA genes of transposon-like intervening sequences that encode ribonuclease III cleavage sites (Burgin *et al.*, 1990). These intervening sequences were assumed to be highly volatile evolutionarily and to reside only where they would be physiologically neutral. It is difficult by such a hypothesis to explain the process that would introduce intervening sequences at multiple sites in a large number of rRNA genes. It is possible that *Salmonella* strains possess a specific mechanism for introducing cleavage sites into their rRNA genes and that these sites confer some selective advantages. The fact that all the cleavage sites that we have seen and others have reported (Pavlakis *et al.*, 1979; Jordan *et al.*, 1980; Ware *et al.*, 1985; Campbell *et al.*, 1987; Spencer *et al.*, 1987; Hsu *et al.*, 1990) lie in the 23S rRNA region suggests that cleavage of 23S rRNA confers an advantage that the cleavage of 16S rRNA does not. Unlike *Salmonella* strains, all *Brucella* and *Agrobacterium* strains that we examined have the same pattern of fragmented 23S rRNA. *Rhodobacter*, which belongs to the same evolutionary line as *Agrobacterium* (Woese, 1987), has been reported to contain the same pattern of fragmented 23S rRNA, suggesting that the introduction of the extra cleavage sites into *Agrobacterium* and *Rhodobacter* might be an ancient evolutionary event. The observation that some strains of *Salmonella* with extra cleavage sites appear to be homogenetic implies that the change leading to the extra site spread through the presumed seven rRNA operons, suggesting that the extra cleavage confers a selective advantage.

We have found that some *Salmonella* strains degrade 23S rRNA at a much faster rate than 16S rRNA which might be related to 23S rRNA fragmentation. It appears that 23S rRNA is polyphyletic with respect to post-transcriptional processing. This phenomenon is important from the standpoints of (*a*) a mechanism(s) that introduces the cleavage sites into the rRNA, (*b*) the phylogenetic significance of extra cleavage sites, and (*c*) the physiological function or advantage of the extra cleavage site. These questions are being examined further.

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